In vitro Cytotoxic and Thrombolytic Activity of Methanolic Extract of Bauhinia acuminata Leaves

Md. Naimul Islam¹, Md. Abul Barakat Fahad¹, Mohammad Robiul Hossain¹, Mohammad Mamun ur Rashid¹, Md. Reyad-ul-Ferdous²,³*, Mohsina Mukti²

¹Department of Pharmacy, International Islamic University Chittagong, 154/A, College Road, Chittagong-4203, Bangladesh.
²Department of Pharmacy, North South University, Bashundhara, Dhaka-1229, Bangladesh.
³Department of Pharmacy, Progati Medical Institute, Dhaka-1207, Bangladesh.

Abstract
The present study was designed to investigate the cytotoxic and thrombolytic activity. Methanolic extract of leaves of B. acuminata was assessed with the Brine shrimp lethality bioassay used to evaluate cytotoxicity. The methanolic extract of B. acuminata was assessed with human blood to evaluate thrombolytic effect. The extract showed remarkable cytotoxic activity, LC₅₀ value of the extract was 15.41 μg/ml compared to vincristin sulphate. It also evaluated for thrombolytic agent compared to streptokinase. It has the significant thrombolytic effect which was about 10.058%.

These findings demonstrate that the leaves extract of B. acuminata have significance cytotoxic and thrombolytic activities. This plant can be used in further investigation for lead compound isolation and other pharmacological activities.

1 Introduction
Bauhinia acuminata belongs to Fabaceae family, an evergreen large shrub, and distributed in areas of southeast Asia, possibly Indonesia, Malaysia or the Philippines¹. In Bangladesh, it grows in hilly forests of Chittagong and Sylhet. It is two to three meters tall. Leaves with petioles 1.5-4 cm long; blades ovate, broadly ovate or sub orbicular, divided about 1/3 their length, membranous, glabrous adaxially, densely puberulent abaxially, base cordate to rounded, apex of lobes acute. The flowers have fragrant, 8 to 12 centimeters in diameter, with five white petals, ten yellow tipped stamens and a green stigma². The species occurs in deciduous forests and scrub. Numbers of known chemical compounds, including phthalic acid, palmitic acid, three phthalic acid esters, gallic acid, ursolic acid were identified from the leaves of B. acuminata². As a part of our continuing investigation of Bangladeshi medicinal plants, the crude methanol extract of B. acuminata were studied for cytotoxic and thrombolytic activities for the first time.

2 Materials and Methods

2.1. Collection of plant sample

The plant, Bauhinia acuminata was collected in August 2013 from the Batali hill, Laikhan bazaar, Chittagong. A voucher specimen is deposited in the Bangladesh National Herbarium, Mirpur, Dhaka and is tagged with the accession number - 38305.

2.2. Preparation of plant extract

The leaves were initially dried in mechanical drier at 60-70° C. The dried samples were crush with a mechanical grinder and powdered samples were kept in clean closed glass container. During grinding of the sample, the grinder was thoroughly cleaned to avoid contamination with any remnant of previously ground material or other foreign material deposited on the grinder. For the purpose of extraction, 100 gm of the powder were soaked in 500 ml methanol and the process of shaking was performed for 7 days on a shaker machine and manually as well. The plant extract was filtered with filter paper with the help of Buchner funnel. Finally, the filtrate was concentrated by evaporating the solvent using a water bath at a temperature of 40° C. A paste-like deep green colored concentrate was obtained.

2.3. Brine shrimp lethality bioassay
Methanolic extracts of Bauhinia acuminata leaves were evaluated for the cytotoxicity test against Artemia salina in a 1-day in vivo-assay by using method4,5.

2.4. Thrombolytic activity

Methanolic extracts of B. acuminata leaves were evaluated for the thrombolytic activity by the method developed by Daginawala et al. (2006)6 and modified by Kawsar et al. (2011)7 using streptokinase (SK) as the standard. The plant extract (100 mg) suspended in 10 ml of distilled water, and was kept overnight. The soluble supernatant was decanted and filtered through a 0.22-micron syringe filter. For clot lysis, healthy volunteers was used to collect venous blood (500 μl) drawn and distributed in different sterile pre weighed microcentrifuge tube and incubated at 37°C for 45 minutes.

3 Results

3.1 Brine shrimp lethality bioassay (Cytotoxic study)

In brine shrimp lethality bioassay using brine shrimp Nauplii, the Methanolic extract of Bauhinia acuminata leaves showed positive result in comparison with the positive control Vincristine Sulphate & that’s why it can be assumed that extract is pharmacologically active. By plotting the log of concentration (logC) versus percent (%) of mortality for all test samples showed an approximate linear correlation. From the graph, the median lethal concentration (LC50, the concentration at which 50% mortality of brine shrimp nauplii occurred) were determined to check the toxic level of the extract. The crude extract of B. acuminate showed significant cytotoxic activity against brine shrimp nauplii and LC50 value was 15.41μg/ml (Figure 1). As positive control Vincristine Sulphate was used & as negative control DMSO was used to validate the test method.

![Figure 1: Determination of LC50 value for extract of B. acuminata leaves from linear correlation between log of concentrations (LogC) versus percent (%) of mortality](image)

3.2. Thrombolytic activity

Addition of 100 μl SK, a positive control (30,000 I.U.) to the clots along with 90 minutes of incubation at 37°C, showed 75.58% clot lysis. Clots when treated with 100 μl sterile distilled water (negative control) showed only negligible clot lysis (4.31%). The in vitro thrombolytic activity study revealed that B. acuminata showed 10.058% clot lysis. The percentage of weight loss of clot after application of extract solution was taken as the functional indication of thrombolytic activity. 10.058% of clot lysis obtained after treating clots with different herbs and appropriate controls is shown in Figure 2.

![Figure 2: Clot lysis by Streptokinase, water and B. acuminata.](image)

4 Discussions

The average lysis of the blood clot on the basis of this was found 10.058%. This seems like a good result so, I can state that the plant part I choose, possesses good response to coagulation by either any way. This plant can be used in further any investigation. As it was only a preliminary test.

The samples showed different mortality rate at different concentration. The mortality rate of brine shrimp Nauplii was found to be increased with the concentration for the sample. The median lethal concentration (LC50) was calculated. The LC50 leaf of B. acuminata was found 10.058 (μg/ml).

5 Conclusions

The methanolic extracts of B. acuminata showed significant thrombolytic and cytotoxic activities. Potential phytochemicals may isolate from this plant. This plant can be used in further investigation for lead compound isolation and other pharmacological activities to exploit their medicinal and pharmaceutical potentialities.

6 Conflict of interest statement

We declare that we have no conflict of interest.

7 References